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Clonal Diversity of Nosocomial Epidemic *Acinetobacter baumannii* Strains Isolated in Spain▽

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***Acinetobacter baumannii* is one of the major pathogens involved in nosocomial outbreaks. The clonal diversity of 729 epidemic strains isolated from 19 Spanish hospitals (mainly from intensive care units) was analyzed over an 11-year period. Pulsed-field gel electrophoresis (PFGE) identified 58 PFGE types that were subjected to susceptibility testing, *rpoB* gene sequencing, and multilocus sequence typing (MLST). All PFGE types were multidrug resistant; colistin was the only agent to which all pathogens were susceptible. The 58 PFGE types were grouped into 16 clones based on their genetic similarity (cutoff of 80%). These clones were distributed into one major cluster (cluster D), three medium clusters (clusters A, B, and C), and three minor clusters (clusters E, F, and G). The *rpoB* gene sequencing and MLST results reflected a clonal distribution, in agreement with the PFGE results. The MLST sequence types (STs) (and their percent distributions) were as follows: ST-2 (47.5%), ST-3 (5.1%), ST-15 (1.7%), ST-32 (1.7%), ST-79 (13.6%), ST-80 (20.3%), and ST-81 (10.2%). ST-79, ST-80, and ST-81 and the alleles *cpn60-26* and *recA29* are described for the first time. International clones I, II, and III were represented by ST-81, ST-2, and ST-3, respectively. ST-79 and ST-80 could be novel emerging clones. This work confirms PFGE and MLST to be complementary tools in clonality studies. Here PFGE was able to demonstrate the monoclonal pattern of most outbreaks, the inter- and intrahospital transmission of bacteria, and their endemic persistence in some wards. MLST allowed the temporal evolution and spatial distribution of Spanish clones to be monitored and permitted international comparisons to be made.**

Multidrug-resistant *Acinetobacter baumannii* is a bacterium frequently endemic in certain hospital wards and is responsible for numerous nosocomial outbreaks around the world (3, 23, 33). Its great capacity to survive in low-moisture environments and its ability to develop resistance to antimicrobial agents afford *A. baumannii* the possibility of spreading in hospitals. The skin, oropharynx, and digestive tract are the main body areas colonized in hospitalized patients. The risk of colonization and subsequent infection are associated with factors such as the presence of underlying severe illnesses, long-term hospitalization, stays in specific hospital wards, selective antimicrobial pressure, and invasive interventions such as the use of mechanical ventilation or catheters (4, 12, 14, 18).

Nosocomial outbreaks of *A. baumannii* can have their origin in a single reservoir or in multiple contaminated sites (37, 39), and infection can have serious repercussions for patient morbidity and mortality. Patients can acquire the bacterium from an environmental source or from other patients (6, 18).

The clonal study of hospital strains is very important in terms of an understanding of the epidemiology of these outbreaks. The aim of the present work was therefore to analyze the genetic diversity and clonal distribution of epidemic strains of *A. baumannii* isolated from around Spain over a long period of time. Isolates collected during outbreaks at different hospitals were analyzed by pulsed-field gel electrophoresis (PFGE), sequencing of the RNA polymerase β subunit (*rpoB*) gene, and multilocus sequence typing (MLST). The results were then compared. Antimicrobial susceptibility testing was also performed to determine the multidrug resistance phenotypes of these epidemic strains.

MATERIALS AND METHODS

Bacterial strains. Over the 11-year period from 1997 to 2007, an initial 814 *A. baumannii* strains isolated from clinical settings and suspected of being involved in nosocomial outbreaks were sent to the Spanish National Center for Microbiology (CNM) for typing. Strains were isolated from 19 public hospitals in 17 Spanish provinces. Hospitals were coded H1 to H19, and provinces were coded P1 to P17. The type of clinical sample, the hospital ward of origin, and the isolation period were registered for all strains (Table 1). Biochemical identification of the strains was undertaken using different commercial systems at the clinical laboratories of origin and was confirmed in our laboratory with Biolog GN2 panels with 95 carbon sources (Biolog, Hayward, CA). For studies based on genetic amplification, DNA was extracted by the boiling method.

PFGE analysis. Plug preparation, lysis, cell washing, restriction digestion, and electrophoresis were performed as previously described (32), with slight differences. The entire chromosomal DNA of the strains was digested with 60 U of ApaI (Takara, Shiga, Japan). PFGE was performed by using a clamped homogeneous electric field electrophoresis (CHEF) DRIII apparatus (Bio-Rad Laboratories, Hercules, CA). The conditions employed were as follows: temperature of 14°C, voltage of 6 V/cm, run time of 28 h, and switch time of 1 to 35 s. The images obtained were processed by using Quantity One v. 4.6.1 software (Bio-Rad).

A PFGE type was considered epidemic when it was isolated from ≥4 patients during an outbreak at a hospital. This was reduced to ≥2 patients for coexisting PFGE types with very closely related profiles. A strain was considered epidemic when it showed an epidemic PFGE type. Thus, 729 epidemic strains were identified among the 814 initial strains studied. In the study of the genetic relationships among the strains, differences in just one band in the PFGE pattern were interpreted as reflecting different PFGE types (34). PFGE clustering was determined by using the unweighted-pair group method with arithmetic averages

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TABLE 1. Clinical and epidemiological data for epidemic *A. baumannii* PFGE types

Hospital/province (no. of beds)	No. of clinical samples									Epidemic PFGE type (no. of strains)	Clone	Hospital ward(s)[b]	Isolation period(s) (mo/yr)
	Respiratory	Wound	Catheter	Urine	Blood	Sterile fluid	Other	Colonization samples[a]	Not informed				
H1/P1 (1,000–1,250)	56	11	14	2	0	0	2	4	0	Ab49 (9)	D6	ICU	05/2007–12/2007
										Ab50 (68)	D6	ICU	06/2007–10/2007
H2/P1 (250–500)	15	7	1	5	0	0	2	2	1	Ab51 (30)	D6	NI	11/2006–07/2007
H3/P2 (<250)	20	5	1	0	2	1	0	0	0	Ab41 (18)	D3	NI	07/2002–11/2003
										Ab39 (2)	D3	NI	08/2002–09/2002
										Ab40 (2)	D3	NI	08/2002, 08/2003
										Ab54 (4)	E	NI	08/2003–11/2003, 10/2004
H4/P3 (750–1,000)	32	8	7	5	11	3	8	9	0	Ab18 (36)	B	ICU	02/2005–02/2007
										Ab13 (5)	B	ICU, IM	03/2005, 02/2006
										Ab29 (4)	D1	NI	03/2005
										Ab9 (5)	B	ICU	06/2005, 02/2006–02/2007
										Ab17 (3)	B	ICU	02/2006
										Ab11 (2)	B	ICU	02/2006–06/2006
										Ab16 (2)	B	ICU	02/2006–06/2006
										Ab19 (8)	B	ICU, IM	02/2006–06/2006
										Ab10 (14)	B	ICU	02/2006–02/2007
H5/P3 (250–500)	26	2	11	4	8	0	0	0	0	Ab9 (28)	B	ICU	07/2005–10/2005, 10/2006, 05/2007
										Ab12 (11)	B	ICU	08/2005–10/2005, 11/2006
										Ab14 (3)	B	ICU	09/2005–10/2005
										Ab15 (9)	B	NI	10/2006–12/2006
H6/P4 (1,500–1,750)	64	1	0	2	0	0	0	77	2	Ab26 (108)	D1	ICU, BU, PSR	03/1999–12/1999, 02/2001
										Ab27 (27)	D1	ICU, BU	07/1999, 12/1999, 02/2001
										Ab38 (3)	D3	ICU, PSR	06/1999, 02/2001
										Ab37 (2)	D1	BU	08/2000
H7/P5 (250–500)	17	19	0	3	1	0	4	6	1	Ab43 (4)	D4	ICU	11/1997–04/1998
										Ab44 (25)	D4	NI	08/2003–01/2005
										Ab31 (4)	D1	NI	11/2003–01/2004
										Ab7 (4)	A2	Traumatology	03/2007–06/2007
H8/P6 (500–750)	4	0	1	2	1	1	2	12	0	Ab8 (5)	A2	ICU	08/2004–02/2005
										Ab5 (6)	A2	ICU	08/2004–03/2005
										Ab6 (11)	A2	ICU	02/2005–03/2005
H9/P7 (750–1,000)	0	0	0	5	0	0	1	0	7	Ab46 (9)	D5	NI	04/2000–05/2000
H10/P8 (1,250–1,500)	7	4	1	0	4	0	0	0	1	Ab30 (12)	D2	ICU	09/2003–11/2003
H11/P9 (250–500)	12	4	0	1	1	0	0	1	0	Ab23 (10)	C	ICU	10/2001–01/2002
										Ab24 (6)	C	ICU	11/2001–01/2002
										Ab25 (2)	C	ICU	12/2001
H12/P10 (250–500)	0	0	0	0	0	0	0	0	11	Ab21 (11)	C	NI	06/2002
H13/P11 (1,250–1,500)	5	0	1	0	3	0	0	20	0	Ab20 (24)	C	ICU	06/1999, 04/2000
										Ab22 (4)	C	ICU	06/1999, 04/2000

H14/P12 (250–500)	10	2	1	0	4	1	2	2	0	Ab56 (12)	G1	ICU	12/2000
H15/P13 (250–500)	3	1	1	0	2	0	2	2	1	Ab28 (7)	D1	ICU	07/1997
										Ab33 (2)	D1	Surgery, ICU	07/1997
										Ab36 (2)	D1	PHC	07/1997
H16/P14 (250–500)	8	1	2	0	0	1	1	0	0	Ab3 (4)	A1	NI	04/2007–07/2007
										Ab4 (2)	A1	NI	05/2007
										Ab1 (2)	A1	NI	06/2007
										Ab2 (2)	A1	NI	06/2007
H17/P15 (750–1,000)	3	0	0	1	0	1	3	2	0	Ab55 (10)	F	ICU, PSR, surgery	04/2007
H18/P16 (250–500)	12	2	1	3	1	1	0	26	6	Ab45 (14)	D4	NI	03/2001–07/2001, 05/2002
										Ab57 (7)	G2	NI	06/2001–08/2001
										Ab42 (2)	D3	NI	11/2001–01/2002
										Ab52 (11)	D7	NI	11/2001–05/2002
										Ab53 (8)	D7	ICU	10/2006
H19/P17 (1,250–1,500)	22	25	9	11	2	2	5	31	4	Ab34 (46)	D1	Cardiology, ICU	04/2007–10/2007
										Ab32 (17)	D1	PHC	05/2007–10/2007
										Ab47 (6)	D5	Cardiology	08/2007–10/2007
										Ab48 (19)	D5	Cardiology, ICU	08/2007–10/2007
										Ab58 (14)	G3	Cardiology	08/2007–10/2007
										Ab35 (2)	D1	Traumatology	09/2007

[a] Nasal, axillary, and rectal exudates for *A. baumannii* screening.
[b] ICU, intensive care unit; NI, not informed; IM, internal medicine; BU, burn unit; PSR, postsurgery room; PHC, primary health care.

(UPGMA) and by using Dice's coefficient. The tolerance was set at 0.8%. All calculations were performed by using InfoQuest software (Applied Maths, Saint-Martens-Latem, Belgium).

Antimicrobial susceptibility. Antimicrobial susceptibility testing of the different PFGE types was undertaken by using two commercial microdilution methods: Phoenix 100 (Becton Dickinson, Shannon, County Clare, Ireland) and MicroScan NM31 panels (Dade Behring, West Sacramento, CA). The MIC of imipenem was also determined by using the Etest (AB Biodisk, Solna, Sweden). Disk diffusion was used to determine susceptibility to sulbactam (SUL), doxycycline (DOX), minocycline (MIN), tigecycline (TIG), and colistin (COL) (all from Oxoid, Basingstoke, Hants, United Kingdom). Results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) criteria for *Acinetobacter* spp. Owing to the lack of standardization for *A. baumannii*, *Enterobacteriaceae* breakpoints were used for interpreting the results of the tigecycline assays. The control strain used was *Escherichia coli* ATCC 25922.

***rpoB* genotyping.** The amplification and sequencing of the 455-bp *rpoB* partial gene were performed for all PFGE types as previously described (20). The *rpoB* gene fragment studied comprises nucleotides 3322 to 3776 in *A. baumannii* type strain ATCC 19606; this was different from the fragment studied by MLST.

MLST. In agreement with the MLST instructions provided at the website of the Institute Pasteur (www.pasteur.fr/recherche/genopole/PF8/mlst/Abaumannii.html), internal fragments of seven housekeeping genes (*cpn60*, *fusA*, *gltA*, *pyrG*, *recA*, *rplB*, and *rpoB*) were amplified and sequenced for each epidemic PFGE type. In the absence of amplification, *rpoB* primers were replaced by *rpo*B+1627 (5′-GGTCCTGGTGGTTTAACACG-3′) and *rpo*B−2231 (5′-CGAATAACGATACGAGAAGCA-3′) on the basis of type strain ATCC 19606. The PCR amplification conditions were those described previously by Bartual et al. (2). The different alleles for each single locus and the allelic profile-associated sequence types (STs) were assigned an identification number. A concatenated locus alignment was used to analyze genetic relationships (21).

Sequencing and data analysis. The purification of the PCR products of the *rpoB* gene and the seven MLST genes was performed by using the QIAamp DNA minikit (Qiagen GmbH, Hilden, Germany). The sequencing of purified fragments was performed with a 3730XL sequencer using the BigDye terminator cycle sequencing kit v3.1 (Applied Biosystems, Foster City, CA). The sequences were assembled by using Lasergene SeqMan II software (DNA Star, Inc., Madison, WI). Sequences were aligned and amino acids were deduced by using the ClustalW routine included in the Lasergene MegAlign software package v.6.1 (DNA Star, Inc.). MLST similarities between allelic profiles were sought by UPGMA using MEGA v. 4 software (K. Tamura, J. Dudley, M. Nei, and S. Kumar, 2007). MLST polymorphic analysis was performed by using DnaSP v. 5.10 software (www.ub.edu/dnasp/).

Nucleotide sequence accession numbers. The three newly identified *rpoB* gene sequences detected were assigned GenBank accession numbers GU942482 to GU942483 and GU942485; the newly identified alleles *cpn60-26* and *recA29* were assigned accession numbers GU942486 and GU942487, respectively.

RESULTS

Bacterial strains. The clinical distribution of the 814 strains was as follows: respiratory tract, 38.8%; nasal, axillary, and rectal samples (from colonization screenings), 23.8%; wounds, 11.3%; catheter tips, 6.3%; urine, 5.4%; blood, 4.9%; sterile fluid, 1.4%; other locations, 3.9%; and not informed, 4.2%. The mean number of strains sent from hospitals was 42.8 (range, 10 to 146). These strains were collected mainly from patients hospitalized in intensive care units (Table 1).

PFGE analysis. Fifty-eight epidemic PFGE types were detected among the 729 epidemic strains and were coded Ab1 to Ab58. The discriminatory power of the technique was expressed via the Simpson diversity index (DI), which showed a value of 0.95 (16).

Table 1 shows the distribution of the PFGE types in the different hospitals (number of strains, hospital wards, and isolation period for each PFGE type). The mean number of PFGE types detected in the studied hospitals was 3 (range, 1 to 9).

Figure 1 shows the clustering of the PFGE types and the hospitals of origin. For all the PFGE types detected, the ge-

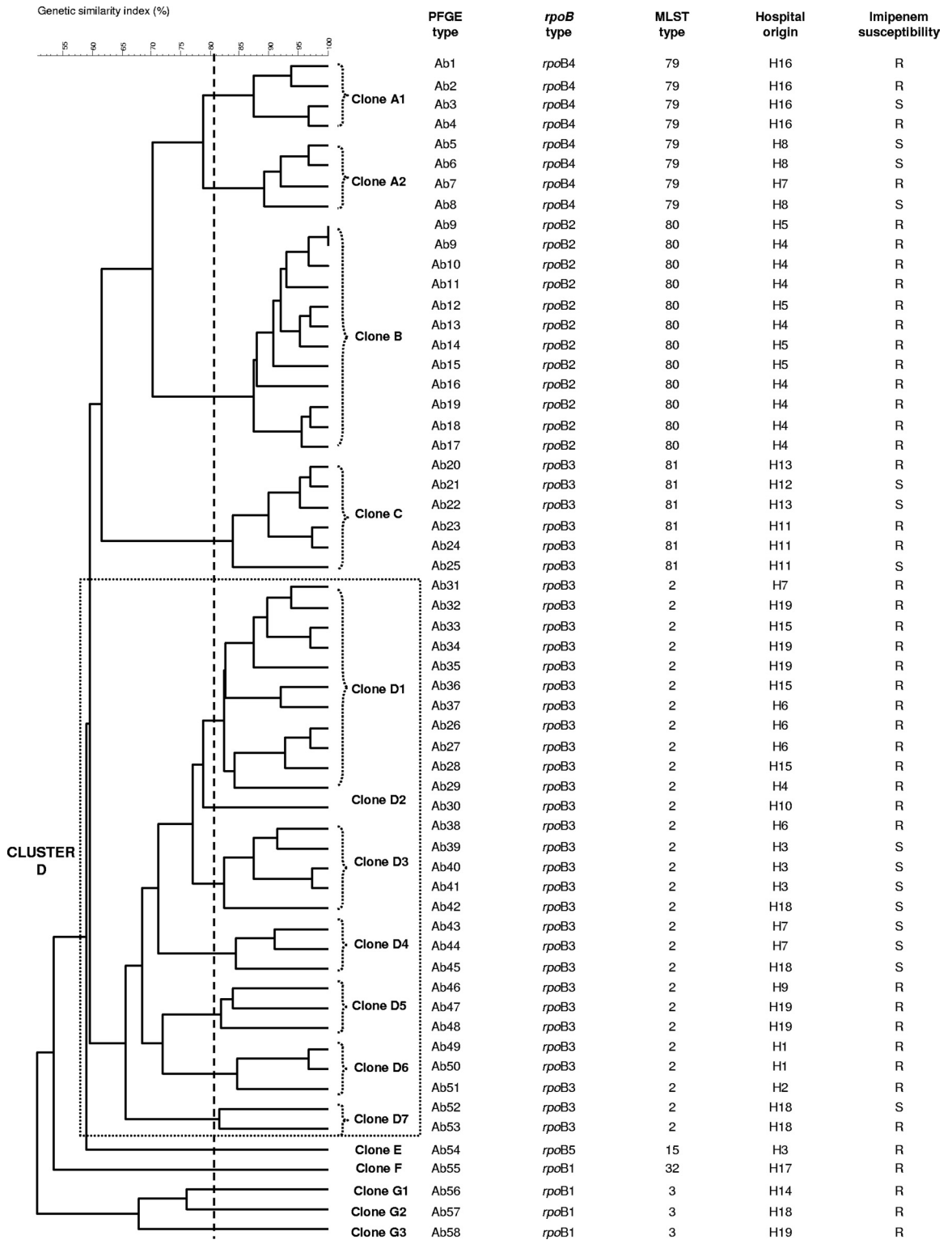

FIG. 1. Dendrogram showing the genetic diversity of *A. baumannii* strains determined by PFGE, *rpoB* gene sequencing, and MLST; the hospital distribution; and the susceptibility to imipenem. The broken line corresponds to the cutoff level (80%) used to define single PFGE clones. Dotted squares mark the boundaries of cluster D. R, resistant; S, susceptible.

netic similarity coefficient ranged from 50% to 100%; PFGE types were considered to represent the same clones when the genetic similarity was >80% (35, 36, 38). Clone A (clones A1 and A2), B, and C grouped 8, 11, and 6 PFGE types, respectively, with genetic similarity ranges of 87 to 97%, 87 to 100%, and 83 to 97%, respectively. For clone B, Ab9 was shared by two different epidemic strains from hospitals H4 and H5. Cluster D grouped 28 PFGE types, containing seven clones (clones

TABLE 2. *rpoB* types of the Spanish *A. baumannii* epidemic strains

rpoB type	Nucleotide and change at position[a]:						No. of positive isolates/total no. of isolates (%)	GenBank accession no.	Reference
	3523	3577	3591	3649	3678	3700			
rpoB1	C	A	G	C	G	C	4/59 (6.8)	DQ207471	20
rpoB2	—	—	**T**	—	—	—	12/59 (20.3)	GU942482	This study
rpoB3	—	—	—	—	**T**	—	34/59 (57.6)	CP000863	17
rpoB4	**T**	—	—	—	**T**	—	8/59 (13.6)	GU942483	This study
rpoB5	—	—	—	**T**	—	**T**	1/59 (1.7)	GU942485	This study

[a] With reference to the *rpoB1* sequence, which corresponds to *A. baumannii* ATCC 19606. Changes are shown in boldface type. —, no changes.

D1 to D7), and the genetic similarity coefficient ranged from 65 to 97%. Clones E, F, and G1 to G3 showed a greater genetic variability among one another than the other clones (50 to 76%).

Antimicrobial susceptibility. Isolates of all PFGE types had a multiple-antimicrobial-resistant phenotype. All isolates were fully resistant to ticarcillin, piperacillin, piperacillin-tazobactam, aztreonam, cefotaxime, ceftazidime, gentamicin, ciprofloxacin, and trimethoprim-sulfamethoxazole. The rates of susceptibility to other antimicrobials were 20.3% for sulbactam (SUL), 1.7% for cefepime, 25.4% for imipenem (the clonal distribution is shown in Fig. 1), 6.8% for meropenem, 10.2% for amikacin, 5.1% for tobramycin, 55.9% for minocycline (MIN), 49.2% for doxycycline (DOX), and 1.7% for tigecycline (TIG). All of the strains were susceptible to colistin (COL). Two main phenotypes were observed: COL susceptible (32.2% of the epidemic PFGE types) and DOX-MIN-COL susceptible (33.9%). Others phenotypes detected included SUL-COL (10.2%), SUL-DOX-MIN-COL (10.2%), MIN-COL (6.8%), and DOX-MIN-TIG-COL (1.7%) susceptible.

***rpoB* genotyping.** The taxonomic identification of the epidemic strains was confirmed by *rpoB* gene sequencing. Five different *rpoB* genotypes were obtained among 59 PFGE types, with a Simpson DI of 0.6. By extrapolating these results (the same PFGE type corresponds to the same *rpoB* gene) to the 729 epidemic strains, the DI was 0.45. The maximum genetic divergence rate was 0.88% The *rpoB1* sequence was identical to that of reference strain *A. baumannii* ATCC 19606. *rpoB3* was the most common sequence (57.6%). *rpoB2*, *rpoB4*, and *rpoB5* are reported here for the first time. A total of five mutations were detected, all of them silent (Table 2). Figure 1 shows the clonal distribution of the *rpoB* types. *rpoB1* appeared in clones F and G1 to G3, *rpoB2* appeared in clone B, *rpoB3* appeared in clones C and D1 to D7, *rpoB4* appeared in clones A1 and A2, and *rpoB5* appeared in clone E.

MLST. Seven different STs were identified among 59 PFGE types, with a Simpson DI of 0.7. By extrapolating these results (the same PFGE type corresponds to the same ST) to the 729 epidemic strains, the DI was 0.48. The number of alleles and polymorphic sites identified for each locus were 5 and 7, respectively, for *cpn60*; 4 and 3 for *fusA*, respectively; 4 and 3 for *gltA*, respectively; 2 and 1 for *pyrG*, respectively; 5 and 7 for *recA*, respectively; 4 and 3 for *rplB*, respectively; and 4 and 3 for *rpoB*, respectively. Two newly identified alleles were described and designated *cpn60-26* (69-T→A) and *recA29* (252-T→C); the *cpn60-1* and *recA3* alleles are the respective reference sequences. A total of 27 polymorphic sites were detected in the concatenated alignment. The maximum genetic divergence rate between STs was 0.57%. Except for the *fusA6* allele, which showed a change in the amino acid sequence, 202-Gly(GGT)→Ser(AGT), all nucleotide changes detected were silent.

The different STs identified were ST-2, ST-3, ST-15, ST-32, ST-79, ST-80, and ST-81. ST-81, ST-2, and ST-3 correspond to international clonal complex 1, clone II, and clone III, respectively (9). ST-79, ST-80, and ST-81 are described here for the first time. ST-79 had two newly identified alleles, *cpn60-26* and *recA29*. For ST-80 and ST-81, the novelty was not any new allele but a new combination of the previously described alleles of the seven housekeeping genes. These newly identified alleles and STs were codified by the Institut Pasteur's MLST *A. baumannii* working group.

The clonal distribution of the STs was as follows (Fig. 1): ST-2 (47.5%) in clones D1 to D7, ST-3 (5.1%) in clones G1 to G3, ST-15 (1.7%) in clone E, ST-32 (1.7%) in clone F, ST-79 (13.6%) in clones A1 and A2, ST-80 (20.3%) in clone B, and ST-81 (10.2%) in clone C. The temporal and geographical distributions of Spanish clones are shown in Fig. 2.

DISCUSSION

Multidrug-resistant *A. baumannii* is one of the major pathogens involved in nosocomial outbreaks. Most of the 814 *A. baumannii* strains sent to the CNM for clonal study came from hospital wards where critical care was provided, such as burn units, postsurgical units, and, in particular, intensive care units (12). The majority of isolates came from respiratory samples, as reported previously for similar studies (Table 1). Critically ill patients have many risk factors that leave them particularly vulnerable to *A. baumannii* infection (4). Indeed, certain invasive life-maintaining interventions, such as respiratory intubation, provide an entry route for many nosocomial pathogens, including *A. baumannii*.

Multidrug-resistant patterns are common in *A. baumannii* nosocomial outbreaks (13, 40). Susceptibility testing of the 58 epidemic PFGE types showed a widespread multidrug-resistant phenotype. All strains were resistant to at least four different antimicrobial groups with known therapeutic activity against *A. baumannii*. The intermediate MICs and halo diameter values obtained were also considered to reflect resistance. The first choice for the treatment of multidrug-resistant *A. baumannii* infection is colistin. This organism shows a very low level of resistance to this agent; indeed, in the present study, full susceptibility was seen.

As for many other bacterial species, PFGE is considered the most discriminatory typing method for *A. baumannii* (16, 25,

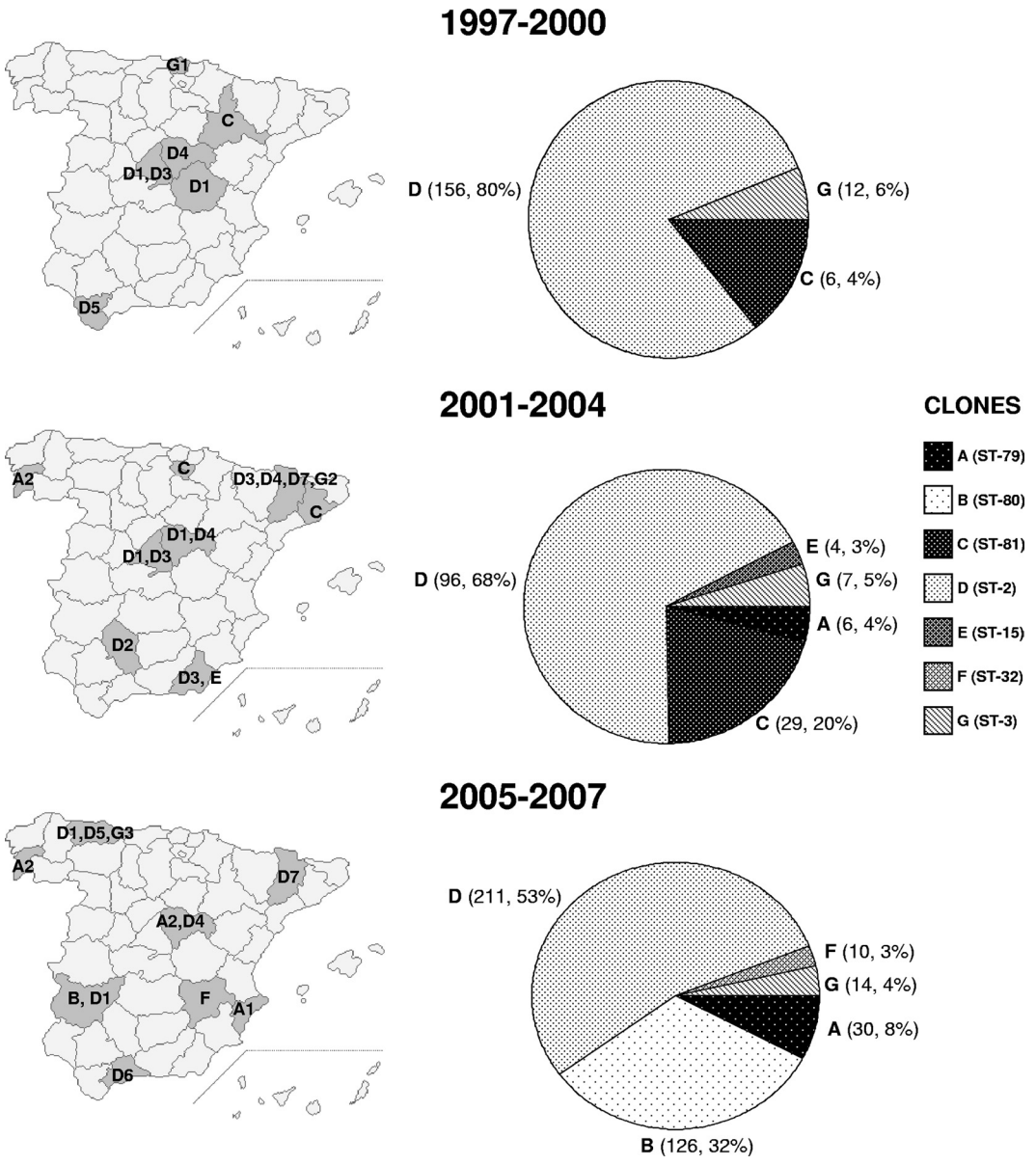

FIG. 2. Spanish *A. baumannii* clones in three time periods. Left maps illustrate its provincial distribution. Right pie charts show the numbers of strains isolated and the global percentage for each clone. (Adapted from a map available at http://www.xtec.net under a Creative Commons license.)

31). In the present study, the high Simpson DI (0.95) showed PFGE to be an efficient tool for determining the genetic relationship between strains isolated in epidemic outbreaks. It was also useful for determining the involvement of the strains in different epidemiological situations (36).

The hospital clonal distribution of the PFGE types revealed two outbreak models: monoclonal and polyclonal (Table 1 and Fig. 1). The monoclonal model was the most common, appearing in 15 out of 19 hospitals. These monoclonal outbreaks were caused by either one epidemic PFGE type (six hospitals) or more than one PFGE type (eight hospitals). In the latter case, one PFGE type usually prevailed over the others. Hospital H7 was affected by different clones that caused four independent monoclonal outbreaks at different times. The polyclonal model affected only four hospitals (H3, H4, H18, and H19). For hospitals H3 and H4, one of the coexisting clones was clearly in the minority. This might reflect the coexistence of sporadic and epidemic clones (30). Hospital H19 had three clones (clones D1, D5, and G3) isolated from the cardiology ward, which might reflect a low level of nosocomial infection control in this unit. This hospital showed a polyclonal pattern (unfortunately, the origin of the outbreak was unknown) plus the intrahospital dissemination of some clones (clones D1 and D5) by a suspected cross-contamination mechanism (24). Hospital H18 showed a polyclonal outbreak with coexisting epidemic and sporadic clones, but in the absence of sufficient information no epidemiological analysis was possible.

The time distribution of the 58 PFGE types showed all of

them to be involved in epidemic outbreaks, although some showed long-term persistence in some hospital wards. In hospital H7, clone D4 was detected over two separate periods of 6 and 17 months. In hospital H18, clone D7 was isolated in two different outbreaks in 2001 and 2006. The reappearance of previously isolated clones in these two hospitals reveals the endemic persistence of *A. baumannii* strains in the nosocomial environment, where they represent a risk factor for future outbreaks.

The interhospital transmission of *A. baumannii* is known to be possible (1, 5, 22). The present study reflects the time distribution of the PFGE types in clones B and D6. Clone B was exclusive to hospitals H4 and H5, both in the same Spanish province, province P3. The genetic similarity of the PFGE types was very high (87 to 100%), with both hospitals sharing type Ab9. This PFGE type was detected for the first time in hospital H4 in June 2005 as a minority type. In hospital H5, type Ab9 was detected in July 2005, the first and major PFGE type isolated in this hospital (26). These data point to the transmission of type Ab9 from hospital H4 to hospital H5, originating a new outbreak in the latter hospital. An unconfirmed explanation for this could be the transfer of patients and/or health care workers from one hospital to another; such an exchange would be favored by their belonging to the same public health area. In hospitals H1 and H2, clone D6 also showed a provincial distribution (province P1) for PFGE types Ab49, Ab50, and Ab51. Although these hospitals share no common PFGE type, an interhospital transmission of the bacteria may still have occurred.

rpoB gene sequencing was tested as a means of obtaining a screening marker that could offer more rapid results for outbreak analysis. The *rpoB* types showed a clonal distribution correlated with the PFGE types (one clone corresponding to one *rpoB* type). Conventional biochemical tests are not able to identify *A. baumannii* with 100% accuracy. In most cases, they are able to assign bacteria only to the *Acinetobacter baumannii-A. calcoaceticus* complex. In this context, the partial sequencing of the *rpoB* gene is a useful tool for identifying bacteria to the species level (19, 20). All the present PFGE types were identified as being *A. baumannii* types by using this technique.

MLST analysis of the epidemic strains returned seven STs (Fig. 1) with a clonal distribution that correlated with the PFGE types (one clone correlated with one ST). The international clonal relationships were made according to an analysis described previously by Diancourt et al. (9). ST-2 corresponds to international clone II. It has been described as being the most common ST in Mediterranean countries (7, 11, 15) and was the most prevalent ST in the present study (47.5%). ST-2 was shared by all clones of cluster D (clones D1 to D7), which were widely distributed across the Spanish provinces examined during the period of 1997 to 2007, accounting for more than 50% of isolates (Fig. 2). ST-3 (5.1%) corresponds to international clone III and appeared in cluster G (clones G1 to G3). The cluster G clones, although minority clones, had a sporadic time distribution throughout the study period (detected in 2000, 2001, and 2007) and showed a random geographical distribution. ST-15 (1.7%) and ST-32 (1.7%) had limited temporal and geographic distributions.

ST-79, ST-80, and ST-81 are described here for the first time. ST-81 (10.2%) differed from ST-1 by a single allele; it was therefore included in clonal complex 1, which comprises international clone I. ST-81 was shared by clone C and had a significant number of isolates in 1999 to 2002 in the northeast of Spain, but since then, it has never again been detected. ST-79 (13.6%) had two newly described alleles (*cpn60-26* and *recA29*) detected in the cluster A clones (clones A1 andA2). The cluster A clones were first isolated in province P6 in 2004 as minority clones. Since 2005, the number of isolations of cluster A clones has increased, spreading to other provinces. ST-80 (20.3%), a new combination of previously described alleles, was shared by clone B isolates. It was detected only in province P3 in 2005 to 2007 and was implicated in the interhospital transmission of *A. baumannii* (from hospital H4 to hospital H5). ST-79 and ST-80 might be novel emerging clones in an expansion process. Both STs should be monitored by MLST to determine their evolution.

International clonal complex 1 (ST-81), clone II (ST-2), and clone III (ST-3) have been repeatedly associated with multidrug-resistant isolates (10, 11, 15, 27, 38). The imipenem-susceptible isolates showed no homogeneous distribution; rather, they showed a biased distribution in cluster A (ST-79), clone C (ST-81), and clones D3, D4, and D7 (all of them of ST-2) (Fig. 1). The ST imipenem susceptibility results fit well with previously reported data (9) for ST-2 (susceptible and resistant strains) and for ST-15 (only resistant strains). ST-3, ST-32, and ST-81, previously described as being imipenem susceptible (9), were resistant in the present work. ST-79 and ST-80 are described for the first time in this study; thus, no previous data are available for comparison.

The study of the genetic variability of *A. baumannii* strains provides much in-depth knowledge regarding their epidemiology. Many hospitals have included this kind of analysis among the measures taken to eradicate outbreaks (8, 15, 26, 29). This work shows that typing studies provide information about time-clonal distribution in different hospital wards and the clonal complexity of outbreaks. The results confirm that a clone can reappear at different times, suggesting that endemic persistence occurs. The results also highlight the intra- and internosocomial transmission of bacteria, reflecting how multidrug-resistant *A. baumannii* has become a hospital problem worldwide.

In conclusion, the present work shows PFGE and MLST to be concordant and complementary tools when used in clonality studies. PFGE offers exhaustive information for outbreak studies, which is useful in local settings and even in nationwide comparisons (28). MLST is a portable typing method (21) that allows interlaboratory comparisons and the large-scale monitoring of the growing list of national and international epidemic clones involved in nosocomial outbreaks (9, 10, 11, 15, 28, 38).

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